

TC299423, A Novel Partial Agonist for Nicotinic Acetylcholine Receptors

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Supplementary information

S1 Table. Brain and Plasma concentrations of TC299423 measured by [¹²⁵I]epibatidine displacement

Brain measurements

<i>Time, min</i>	<i>K_i, pM</i>	<i>[TC299423] in assay, nM</i>	<i>[TC299423] in brain, pmol/mg^a</i>
Control	23 ± 2.3	0	0
5	27 ± 2.5	2.2 ± 1.1	45 ± 25
10	31 ± 1.7	4.4 ± 0.9	88 ± 18
20	29 ± 0.4	3.7 ± 0.2	73 ± 4.0

Blood measurements

<i>Time, min</i>	<i>Vol serum for IC₅₀, µL</i>	<i>[TC299423] nM</i>
5	42 ± 9.7	250 ± 50
10	28 ± 5.5	370 ± 70
20	89 ± 18	120 ± 30

Data are expressed as mean ± SEM

^a, Data are pmol TC299423 / mg of brain tissue

S2 Table. *In vitro* metabolism of TC299423, compared with varenicline.

	Human microsomes		Rat microsomes		Turnover by human cytochrome P450 isoforms					
	Clearance ^a	T _{1/2} ^b	Clearance ^a	T _{1/2} ^b	CYP1A2 ^c	CYP2C9 ^c	CYP2C19 ^c	CYP2D6 ^c	CYP3A4 ^c	Human FMO3 ^c
Varenicline	39	43	233	10	7	0	0	39	50	
TC299423	5	285	18	79	3	8	4	42	7	17

The assay mixtures (final incubation volume of 125 µL) in 0.255 M phosphate buffer with 0.575% (w/v) KCl (pH 7.4) contained PiB (1.0 µM), 2.0 mM nicotinamide-adenine dinucleotide phosphate (reduced form) (NADPH), and a recombinantly expressed cytochrome P450 enzyme preparation (1A2, 2C9, 2C19, 2D6, 3A4, and FMO3) at 100 pmol/mL. After a 10 min preincubation at 37 °C, the reaction was initiated by addition of 2.0 mM NADPH and incubated for 60 min. The reactions were terminated by the addition of two volumes of DMSO. Samples were subsequently centrifuged at 4,000 rpm for 10 min. From the resulting supernatant, 10 µL was analyzed by liquid chromatography/tandem mass spectrometry (LC-MS/MS). Control experiments were performed by substituting the active enzyme preparation by cell preparations containing no recombinant human CYP.

^a, Data for clearance are given as µL/min/mg protein.

^b, Unit for T_{1/2} is min.

^c, Data for cytochrome P450 turnover and for human flavin-containing monooxygenase 3 (FMO3) are given as percent decline over 60 min.

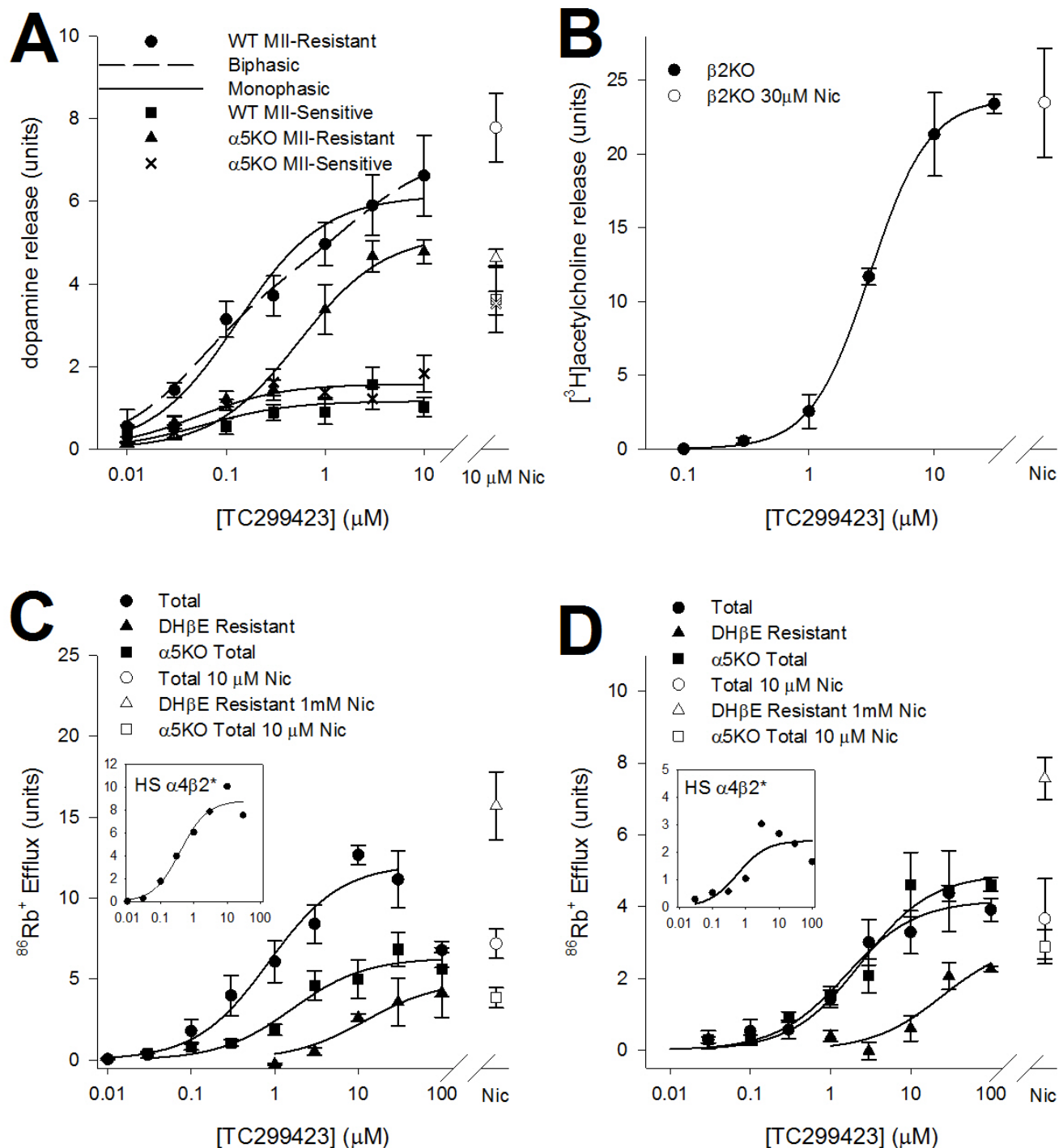
S3 Table. Competition binding on 70 target proteins tested at a concentration of 1 μ M TC299423

Inhibition	Assay Name	Radioligand/Substrate	Hit
12.86%	Adenosine Transporter (h)	[3H]-NBTI	FALSE
7.85%	Adenosine, A1	[3H]CPX	FALSE
1.51%	Adenosine, A2A (h)	[3H]CGS 21680	FALSE
-0.05%	Adrenergic, Alpha 1A	[3H]-7-MeOxy-Prazosin	FALSE
-8.31%	Adrenergic, Alpha 1B	[3H]-7-MeOxy-Prazosin	FALSE
6.75%	Adrenergic, Alpha 2A (h)	[3H]MK-912	FALSE
-14.09%	Adrenergic, Alpha 2B	[3H]MK-912	FALSE
6.28%	Adrenergic, Alpha 2C (h)	[3H]MK-912	FALSE
11.21%	Adrenergic, Beta 1 (h)	[125I] (-) Iodocyanopindolol	FALSE
-8.25%	Adrenergic, Beta 2 (h)	[125I]-Iodo-cyanopindolol	FALSE
-5.87%	Dopamine Transporter	[3H]WIN 35,428	FALSE
-2.94%	Dopamine, D1 (h)	[3H]-SCH23390	FALSE
-21.17%	Dopamine, D2s (h)	[3H]-Raclopride	FALSE
-16.93%	Dopamine, D3	[3H]7-OH-DPAT	FALSE
-2.44%	Dopamine, D4.4 (h)	[3H]-YM-09151-2	FALSE
-2.66%	GABA A, Agonist Site	[3H]GABA	FALSE
11.84%	GABA A, BDZ, alpha 1 site	[3H]Flunitrazepam	FALSE
-2.43%	GABA-B	[3H]CGP 54626A	FALSE
-1.26%	Glutamate, AMPA Site (Ionotropic)	[3H]AMPA	FALSE
-6.93%	Glutamate, Kainate Site (Ionotropic)	[3H]Kainic acid	FALSE
5.46%	Glutamate, MK-801 Site (Ionotropic)	[3H]MK-801	FALSE
5.51%	Glutamate, NMDA Agonist Site (Ionotropic)	[3H]CGP 39653	FALSE
7.71%	Glutamate, NMDA, Phencyclidine Site (Ionotropic)	[3H]TCP	FALSE
4.34%	Glutamate,NMDA,Glycine (Stry-insens Site) (Ionot	[3H]-MDL-105,519	FALSE
-7.63%	Glycine, Strychnine-sensitive	[3H]Strychnine	FALSE
15.56%	Histamine, H1	[3H]Pyrilamine	FALSE
6.50%	Histamine, H2	[125I]-Aminopotentidine	FALSE

5.85%	Histamine, H3	[3H]N-a-MeHistamine	FALSE
-11.73%	Muscarinic, M1 (hr)	[3H]Scopolamine, N-Methyl	FALSE
-3.18%	Muscarinic, M2 (h)	[3H]Scopolamine, N-Methyl	FALSE
-4.77%	Muscarinic, M3 (h)	[3H]Scopolamine, N-Methyl	FALSE
-6.69%	Muscarinic, M4 (h)	[3H]Scopolamine, N-Methyl	FALSE
2.24%	Muscarinic, M5 (h)	[3H]Scopolamine, N-Methyl	FALSE
93.33%	Nicotinic, Neuronal (α-BnTx insensitive)	[3H]Epibatidine	TRUE
23.72%	Norepinephrine Transporter	[3H]Nisoxetine	FALSE
-2.12%	Opioid, Delta 2 (h)	[3H]-Naltrindole	FALSE
-6.43%	Opioid, Mu (h)	[3H]-Diprenorphine	FALSE
7.03%	Serotonin Transporter	[3H]Citalopram, N-Methyl	FALSE
17.60%	Serotonin, 5HT1A (h)	[3H]-8-OH-DPAT	FALSE
9.99%	Serotonin, 5HT1D	[3H]5-CT	FALSE
12.43%	Serotonin, 5HT2A	[3H]Ketanserin	FALSE
-2.21%	Serotonin, 5HT2C	[3H]Mesulergine	FALSE
7.50%	Serotonin, 5HT3	[3H]GR 65630	FALSE
-16.64%	Serotonin, 5HT4	[3H]GR 113808	FALSE
1.24%	Serotonin, 5HT5A (h)	[3H]-LSD	FALSE
8.87%	Serotonin, 5HT6 (h)	[3H]-LSD	FALSE
-2.10%	Serotonin, 5HT7 (h)	[3H]LSD	FALSE
-0.52%	Sigma 1	[3H]-(+)-Pentazocine	FALSE
-0.65%	Sigma 2	[3H]-DTG	FALSE
-3.91%	Calcium Channel, Type L (Dihydropyridine Site)	[3H]Nitrendipine	FALSE
-0.60%	Calcium Channel, Type N	[125I]-Conotoxin GVIA	FALSE
6.83%	GABA, Chloride, TBOB Site	[3H]TBOB	FALSE
52.12%	Potassium Channel, ATP-Sensitive	[3H]Glibenclamide	TRUE
-2.57%	Potassium Channel, Ca2+ Act., VI	[125I]Apamin	FALSE
14.46%	Potassium Channel, I[Kr] (hERG) (h)	[3H]Astemizole	FALSE
6.91%	Sodium, Site 2	[3H]Batrachotoxin A 20-a-Benzo	FALSE

-10.86%	Nitric Oxide, NOS (Neuronal-Binding)	[3H]NOARG	FALSE
29.86%	Leukotriene, LTB4 (BLT)	[3H]LTB4	FALSE
-8.26%	Leukotriene, LTD4 (CysLT1)	[3H]LTD4	FALSE
3.14%	Thromboxane A2 (h) [SQ 29,548]	3H SQ 29,548	FALSE
24.85%	Angiotensin II, AT1 (h)	[125I]-(Sar1-Ile8) Angiotensin	FALSE
32.29%	Bradykinin, BK2	[3H]Bradykinin	FALSE
8.93%	Endothelin, ET-A (h)	[125I] Endothelin-1	FALSE
2.10%	Neurokinin, NK1	[3H]Substance P	FALSE
2.16%	Neuropeptide, NPY2 (h)	[125I]-PYY	FALSE
21.55%	Esterase, Acetylcholine	Acetylthiocholine	FALSE
e12.00%	Phosphodiesterase, PDE4A1A (h)	Fluorescent cyclic AMP	FALSE
-3.00%	Phosphodiesterase, PDE5A1 (h)	Fluorescent cyclic GMP	FALSE
-5.00%	Kinase, Protein, PKA (h)	Fluorescein-labeled peptide	FALSE
-1.00%	Kinase, Protein, PKCa (h)	Fluorescein-labeled peptide	FALSE

TC299423 inhibited >50% of the reference radioligand for two sites: Neuronal nAChRs and ATP-sensitive potassium channels. 1µM TC299423 inhibited 93.33% of the [³H]-epibatidine binding at nAChRs and 52.12% of the [³H]-Glibenclamide binding at the ATP-sensitive potassium channels.



S1 Figure. Functional assays for measuring TC299423 agonist activity at various nAChRs. All points are mean \pm SEM. **A**) Closed symbols show α -CtxMII-resistant [^3H]-dopamine release and α -CtxMII-sensitive [^3H]-dopamine release from WT and $\alpha 5\text{KO}$ striatal synaptosomes. Open symbols show [^3H]-dopamine release evoked by 10 μM nicotine for the matching closed symbols. **B**) Measurement of functional $\alpha 3\beta 4^*$ -nAChR by [^3H]-ACh release from $\beta 2\text{KO}$ IPN synaptosomes. Curve fit EC_{50} and efficacy values for (A) and (B) are shown in Table 1 (see Results). **C**) Activity of TC299423 on $\alpha 4\beta 2^*$ measured by high-sensitivity $^{86}\text{Rb}^+$ efflux from WT

and $\alpha 5$ KO thalamic synaptosomes. 2 μ M DH β E was used to isolate the high-sensitivity and low-sensitivity responses. **D)** Activity of TC299423 on $\alpha 4\beta 2^*$ measured by high-sensitivity $^{86}\text{Rb}^+$ efflux from WT and $\alpha 5$ KO cortical synaptosomes. As in C, 2 μ M DH β E was used to distinguish between high-sensitivity and low-sensitivity nAChRs. Calculated EC₅₀ and efficacy values for **(C)** and **(D)** are presented in Table 2 (see Results).